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Monitoring Microbial Diversity of a Full-Scale Municipal Wastewater Treatment Plant in Dubai

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Abstract

In this study, fluorescence in situhybridization (FISH) technique was employedfor monitoring the microbial community in the activated sludge system of a full-scale municipal wastewater treatment plant in Dubai over a period of one year. A total of 96 activated sludge mixed liquor samples were characterized using ribosomal RNA (16S and 23S rRNA) targeted oligonucleotide probes for defined phylogenetic groups of bacteria. Several filamentous and non-filamentous bacteriawere predominantly found throughout the study period in all activated sludge mixed liquor samples. The bacterial species belonging to High G+C group were detected in both branched and single cell morphotypes. The previously published genus and species specific probes detected several of members belonging to archaea, sulphate reducing bacteria, ammonia-oxidizing bacteria, nitrobacter, nitrospira including halophilic and halotolerantnitrosomonasspp.In conclusion, the overall microbial community populations detected by the sub-group and species specific 16S and 23S rRNA targeted oligonucleotide probes found to be quite diverse and varied seasonally.

*Keywords:*Fluorescence in situ hybridization; microbial community; activated sludge; oligonucleotide probes

1. Introduction

(Activated sludge systems represent a widely used technology for domestic and municipal wastewater treatment in most countries [1, 2]. Basic understanding of the microorganisms and their activity under different conditions are keys for its successful operation [3]. The health of an activated sludge system thus

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depends upon its microbial diversity, which again is dependent on the influent wastewater, environmental parameters and prevalent operational conditions [4, 5, 6]. Monitoring of the microbial community in such plants can be instrumental in understanding and control of bulking and foaming which are caused chiefly by filamentous bacterial communities [2]. Over recent years, the growth of 16S and 23S ribosomal RNA sequence databases have enabled researchers to use rRNA-targeted hybridization for studying activated sludge biomass. Oligonucleotide probes targeting specific domains, genera, species, or even strains have been developed. Molecular probe based detection techniques like FISH have been successfully employed for this purpose [7, 8].

At present Dubai sewage treatment plant, one of the major wastewater treatment plants in Dubai city is frequently challenged by bulking and foaming episodes in its activated sludge system. In our earlier study [3], various filamentous bacteria were identified and reported on the basis of classical morphological features as described by Jenkins et al. 2003 [2].The accurate identification and quantification of foaming and bulking-causative organisms may guide future activated sludge modeling and the development of rational control measures in the activated sludge units of the sewage treatment plant in Dubai. This investigation aimed at monitoring the microbial community in the activated sludge system of a full-scale municipal wastewater treatment plant in Dubai over a period of one year beginning May 2011. Fluorescence in situ hybridization (FISH) was employed using previously published 16S and 23S ribosomal RNA (rRNA) targeted oligonucleotide probes specific for established phylogenetic groups of bacteria to detect the presence of the different groups and subgroups of bacteria directly within the activated sludge mixed liquor samples. The abundance of these groups was obtained numerically through computer analysis of the images taken when the hybridized samples were examined under the fluorescent microscope.

2. Materials and methods

2.1. Sample collection

250 ml of mixed liquor sludge samples were collected from the aeration tanks of the activated sludge system of a full-scale WTP located at the Al Aweer area in Dubai. Samples were taken on a fortnightly basis spanning over a one year period. Samples were stored at 4°C and fixed within 24 hrs. The samples were fixed both in ethanol and 4% (w/v) paraformaldehyde as described earlier [10].

2.2. Characterization of microbial community

The fluorescence in situ hybridization technique was performed on the activate sludge mixed liquor samples using the methods described earlier [11]. Hybridization with sludge samples was performed by domain, group/class, genus and species specific probes. The list of oligonucleotide probes used in this study and their specificity are described in Table 1 and in our earlier study [9]. All oligonucleotide probes were labeled at their 5' end by tetramethylrhodamineisothiocyanate (TRITC). Briefly, In situ hybridization of activated sludge mixed liquor sampleswas carried out at a constant temperature of 46 °C in 50 ml polypropylene centrifuge tubes as a hybridization chamber. On each well of the microscope slide, 9 µl hybridization buffer (5 M NaCl; 1 MTris-HCl, pH 7±2; 10%, w/v, SDS) and 1 µl of probe (50 nanogram) was applied and incubated for 90-120 minutes. Stringent hybridization conditions for the different oligonucleotide probes were adjusted by different formamide concentrations in the hybridization buffer. Washing buffer(5 M NaCl; 1 MTris-HCl, pH 7±2; 0.5 M EDTA) with varying sodium chloride corresponding to the formamide concentrations was prepared according to the method described by Daims et al. 2005 [11].For microscopic examination the slides were mounted in Citifluor AF1 (Citifluor Ltd., London, UK) and examined with an

epifluorescent microscope (NIKON eclipse 80i, JAPAN) fitted with filter sets G-2E/C (for TRITC) and B-2E/C (for FITC) and a 100W high pressure mercury lamp. FISH images were captured and analyzed using the ProgRes digital camera system (JENOPTIK, Germany).

Table 1.Oligonucleotide probes used in this study

Probe name	Sequence (5` to 3`)	Probe specificity	Formamide (%)	Reference
HGC 69a	TATAGTTACCACCGCCGT	Gram positive high G+C content genus *Gordona*	20	[10]
S-G-Gor- 0596-a-A-22	TGCAGAATTTCACAGACGACGC		20	[13]
S-S-G.am 0192-a-A-18	CACCCACCCCCATGCAGG	*Gordonaamarae*	30	[13]
MNP1	TTAGACCCAGTTTCCCAGGCT	Nocardioform actinomycetes	50	[10]
Myc657	AGTCTCCCCTGYAGTA	Mycobacterium subdivision	30	[14]

3. Results and discussion

All of the 96 mixed liquor samples successfully hybridized by EUB mix (I-III) probes with respect to DAPI. This indicated the presence of highly physiologically active bacterial populations within the sample. Furthermore, morphological examination of mixed liquor sludge samples using methods described by Jenkins et al., 2003[2] also revealed a large number of filamentous bacteria during the current study period and as indicated in our earlier studies [3,9]. There were at least three distinct filamentous bacteria that were detected by GAM42a, HGC69a and Alpha1b probes. The filamentous bacteria targeted by GAM42a and HGC69a were always observed in all of the samples during the period of study. In all of the samples, the Gam42a probe identified long branched irregular filaments whose population remained constant. However, genus and species specific oligonucleotide probes for filamentous gram negative bacteria like Sphaerotilusnatans (SNA), Liptothrixdiscophora (LD1), Leucothrixmucor (LMU), Haliscomenobacter (HHY), Thothrixnivea (TN1) and Eikelboom type 021N (021N) failed to hybridize in the samples. This might be due to low permeability of these bacterialpopulationstospecific oligonucleotide probes by employed fixation procedures as indicated in other studies [10].The oligonucleotide probe Alpha1b identified small branched irregular filaments in at least 10 mixed liquor samples.

Several large cocci in clusters were found in most of the samples targeted by Gamma 42a. Also, there were long and short rods targeted by Gam42a probe, probably Enterobacteriaceae, observed in all of the samples. The oligonucleotide probe Alpha1b identified small cocci in tetrad arrangement and single cell rods probably belonging to alpha-subclass of proteobacteria. However, tetrad cocci were consistently found in at least 20 mixed liquor samples. In a few samples, the Beta 42a probe detected small rods and small cocci of 1-2 μm size existing individually and in clusters. The gram positive bacteria with low G+C (LGC mix probe) targeted mostly spore bearing rods, similar to those described in earlier reports [12] and cocci with a size of 2-3 μm. In at least 20 samples, the LGC mix probe [9] identified long or small rods scattered throughout the sample. The cocci targeted by the LGC probe were found in 18 of the 24 samples. These cocci occurred in clusters or in diplococci/streptococci/staphylococci arrangements. However, quantitatively the LGC probe targeted quite a small percentage of the bacterial population in comparison to GAM42a and HGC69a probes.

The HGC69a probe detected a group of filamentous bacteria that was not targeted by Gamma 42a and Alpha1b. It was noted that the population of this group of gram positive bacteria with high G+C content remained dominant throughout the sampling period as described earlier [3]. This was probably because of the frequent foaming incidences observed in the treatment plant throughout the study period. Most of the bacteria

targeted by HGC69a were either branched filaments or long, medium or small size curved rods. These filamentous morphotypes were found to be dominant in all of the mixed liquor samples. All 24 samples gave positive hybridization with the HGC69a probe indicating that the gram positive bacteria with high G+C content, resembling "nocardiaamarae like organism"[13], was the most significant microbial community in DSTP. This observation suggests that this group significantly influenced the activated sludge process. In situ hybridization using the nocardioform specific MNP1 probe [10] was performed on the samples previously hybridized with HGC69a probe. MNP1 probe was able to detect two morphotypes. One branched filament type representing typical nocardioformactinomycetes and the other one comprising of short irregular rods (Fig. 1 A, B, C). This observation supports an earlier study [10], where MNP1 probe detected similar populations with different morphologies. The previously published genus and species specific probes detected several of members belonging to archaea, sulphate reducing bacteria, ammonia-oxidizing bacteria, nitrobacter, nitrospira including halophilic and halotolerantnitrosomonas spp.

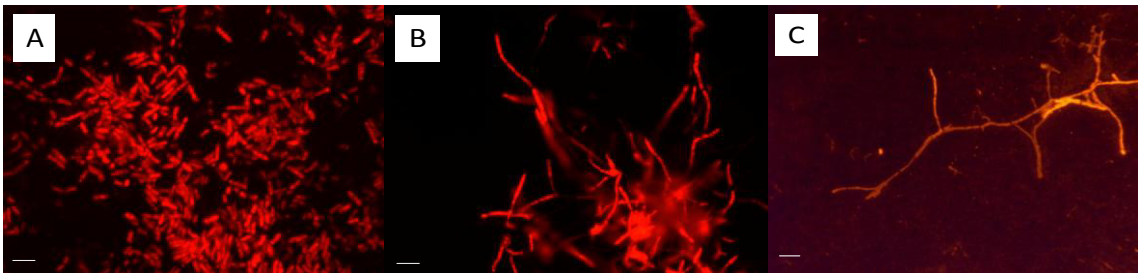

Fig. 1. Nocardioformactinomycetemorphotypes in activated sludge mixed liquor samples hybridized by TRITC–labeledMNP1 probe. A) Non-filamentous B, C) Filamentous.Bar =10 μm and applies to all photomicrographs. Original magnification: 1000X

The samples containing nocardioform populations detected by probe MNP1 and HGC69a were further analyzed by hybridization with the Gordonaamarae and genus Gordona specific probes previously reported by De Los Reyes et al. 1997 [14]. These two probes failed to detect bacterial populations in WTP samples indicating that Gordona genus members were not dominant. However, it is difficult to draw an early conclusion on the absence of this particular bacterial community, as the oligonucleotide probes used in this study were designed for specific studies in other countries were probably not suitable to detect the populations of Gordona genus. It is possible that the bacterial species found in Dubai WTP might be different due to the different geographical distribution affected bylocal environmental conditions in the UAE.

4. Conclusion

This study evaluated the microbial community structure of a full-scale municipal wastewater treatment plant in Dubai. The population changes of the major higher taxonomic groups such as proteobacteria (alpha, beta and gamma), Archaea, Cytophaga, gram positive bacteria with high G+C and low G+C content was evaluated by FISH technique. The previously published genus and species specific probes detected several of members belonging to archaea, sulphate reducing bacteria, ammonia-oxidizing bacteria,nitrobacter, nitrospira including halophilic and halotolerantnitrosomonas spp. Several filamentous bacteria belonging to High G+C group were found to be dominating the activated sludge system throughout the period of study. Further work based on clone library based approaches with newly designed oligonucleotide probes will help to reveal the dynamics of the microbial community in wastewater treatment plants in the United Arab Emirates.

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